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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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15535 U.S.PTO
60/527875

Title: Band5: A Human Testis Specific Protein

10 Sheets of specification.
10 Sheets of drawings.

University of Virginia Patent Foundation claims small entity status as a nonprofit organization (37 CFR §§1.27(a)(3) and (c)). The Commissioner is hereby authorized to charge the Small Entity Fee of **$80** to **Deposit Account No. 50-0423.**

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This invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. The government has certain rights in the invention.

YES ☒ NO ☐ Grant No. NIH U54 HD29099 & HD 38082

Dated: December 8, 2003

Respectfully submitted,

By: _____
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00955-01

Band5: A Human Testis Specific Protein

US Government Rights

This invention was made with United States Government support under Grant Nos. HD 38082, and U54 29099, awarded by National Institutes of Health. The United States Government has certain rights in the invention.

Background

Lipid raft domains are regions of plasma membranes that have distinct lipid content and are enriched in cholesterol and sphingolipids. The uniques content of these domains is believed to recruit specific proteins to the plasma membrane and these domains are implicated in signal transduction. If the protein caveolin is present then the membrane domaine is defined as a caveolae. Caveolins are cholesterol binding proteins that can potentially regulate a variety of signal transduction pathways (Smart et al., (1999) Mol. Cell. Biol. 19, 7289-7304; Kurzchalia & Parton, (1999) Curr. Opin. Cell. Biol. 11, 424-431). Additional uncharacterized proteins are believed to be associated with the lipid raft domains, and since the raft domain dissociates with capacitation these proteins may play key roles in the capcitation process. In accordance with one embodiment of the present invention proteins associated with the lipid raft domains of sperm cells are isolated and characterized.

One aspect of the present invention relates to signaling events in mammalian sperm that regulate the functions of this highly differentiated cell. More particularly, in one embodiment the invention relates to signal transduction that modulates the acquisition of sperm fertilizing capacity. After ejaculation, sperm are able to move actively but lack fertilizing competence. They acquire the ability to fertilize in the female genital tract in a time-dependent process called capacitation. Capacitation has been demonstrated to be accompanied by phosphorylation of several proteins on both serine/threonine and tyrosine residues, and that protein tyrosine phosphorylation is regulated downstream by a cAMP/PKA pathway that involves the crosstalk between these two signaling pathways. With the exception of PKA, the other kinase(s) involved in the regulation of capacitation are still unknown.

Raft fractions can be isolated with reproducibility from mouse caudal sperm using ultracentrigfugation of membranes in a sucrose gradient according to standard techniques known to those skilled in the art (see Fig. 1). As shown in Fig. 2 the proteins present in sucrose fractions of the isolated lipid raft domains isolated

from noncapacitated sperm differ from those isolated from capacitated sperm. Silver stained PAGE analysis reveals that a number of proteins present in the lipid raft domains of noncapacitated sperm are not present in those domains in capacitated sperm, thus raft domains are diminished in protein content (especially true for fraction 4) upon capacitation of the sperm (see Fig. 2).

In accordance with one embodiment of the present invention a sperm raft domain associated protein is isolated and characterized. In accordance with one embodiment, the human and mouse Band 5 genes and proteins serve as a targets for the development of novel drugs, including the identification of novel contraceptive agents.

Summary of Various Embodiments of the Invention

The present invention is directed to the human and mouse Band 5 genes, their respective encoded proteins and antibodies against those proteins. More particularly, the present invention is directed to polypeptides comprising the amino acid sequences disclosed in Figs. 6. Antagonists of Band 5 activity are anticipated to have ustility as contraceptive agents and thus one aspect of the present invention is directed to a method of screening for inhibitors of Band 5. The present invention also encompasses antibodies specific for Band 5 and the use of such antibodies as therapeutic and diagnostic tools.

Detailed Description of Embodiments

Definitions

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, the term "purified" and like terms relate to an enrichment of a molecule or compound relative to other components normally associated with the molecule or compound in a native environment. The term "purified" does not necessarily indicate that complete purity of the particular molecule has been achieved during the process. A "highly purified" compound as used herein refers to a compound that is greater than 90% pure.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and

various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

A polylinker is a nucleic acid sequence that comprises a series of three or more closely spaced restriction endonuclease recognitions sequences.

"Operably linked" refers to a juxtaposition wherein the components are configured so as to perform their usual function. Thus, control sequences or promoters operably linked to a coding sequence are capable of effecting the expression of the coding sequence.

As used herein, "nucleic acid," "DNA," and similar terms also include nucleic acid analogs, i.e. analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention.

The term "peptide" encompasses a sequence of 3 or more amino acids wherein the amino acids are naturally occurring or synthetic (non-naturally occurring) amino acids. Peptide mimetics include peptides having one or more of the following modifications:

1. peptides wherein one or more of the peptidyl --C(O)NR-- linkages (bonds) have been replaced by a non-peptidyl linkage such as a $-CH_2$-carbamate linkage ($--CH_2OC(O)NR--$), a phosphonate linkage, a $-CH_2$-sulfonamide ($-CH_2--S(O)_2NR--$) linkage, a urea ($--NHC(O)NH--$) linkage, a $--CH_2$ -secondary amine linkage, or with an alkylated peptidyl linkage ($--C(O)NR--$) wherein R is C_1-C_4 alkyl;

2. peptides wherein the N-terminus is derivatized to a $--NRR_1$ group, to a -- NRC(O)R group, to a --NRC(O)OR group, to a $--NRS(O)_2R$ group, to a --NHC(O)NHR group where R and R_1 are hydrogen or C_1-C_4 alkyl with the proviso that R and R_1 are not both hydrogen;

3. peptides wherein the C terminus is derivatized to $--C(O)R_2$ where R_2 is selected from the group consisting of C_1-C_4 alkoxy, and $--NR_3R_4$ where R_3 and R_4 are independently selected from the group consisting of hydrogen and C_1-C_4 alkyl.

Naturally occurring amino acid residues in peptides are abbreviated as recommended by the IUPAC-IUB Biochemical Nomenclature Commission as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I;

Methionine is Met or M; Norleucine is Nle; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gln or Q; Asparagine is Asn or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; Glycine is Gly or G, and X is any amino acid. Other naturally occurring amino acids include, by way of example, 4-hydroxyproline, 5-hydroxylysine, and the like.

Synthetic or non-naturally occurring amino acids refer to amino acids which do not naturally occur *in vivo* but which, nevertheless, can be incorporated into the peptide structures described herein. The resulting "synthetic peptide" contains amino acids other than the 20 naturally occurring, genetically encoded amino acids at one, two, or more positions of the peptides. For instance, naphthylalanine can be substituted for trytophan to facilitate synthesis. Other synthetic amino acids that can be substituted into peptides include L-hydroxypropyl, L-3,4-dihydroxyphenylalanyl, alpha-amino acids such as L-alpha-hydroxylysyl and D-alpha-methylalanyl, L-alpha.-methylalanyl, beta.-amino acids, and isoquinolyl. D amino acids and non-naturally occurring synthetic amino acids can also be incorporated into the peptides. Other derivatives include replacement of the naturally occurring side chains of the 20 genetically encoded amino acids (or any L or D amino acid) with other side chains.

As used herein, the term "conservative amino acid substitution" is defined herein as an amino acid exchange within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:
   Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides:
   Asp, Asn, Glu, Gln;

III. Polar, positively charged residues:
   His, Arg, Lys;

IV. Large, aliphatic, nonpolar residues:
   Met Leu, Ile, Val, Cys

V. Large, aromatic residues:
   Phe, Tyr, Trp

As used herein, the term "antibody" refers to a polyclonal or monoclonal antibody or a binding fragment thereof such as Fab, F(ab')2 and Fv fragments.

As used herein, the term "biologically active fragments" or "bioactive fragment" of a tssk polypeptide encompasses natural or synthetic portions of the full-length protein that are capable of specific binding to their natural ligand.

The term "non-native promoter" as used herein refers to any promoter that has been operably linked to a coding sequence wherein the coding sequence and the promoter are not naturally associated (i.e. a recombinant promoter/coding sequence construct).

As used herein, a transgenic cell is any cell that comprises a nucleic acid sequence that has been introduced into the cell in a manner that allows expression of a gene encoded by the introduced nucleic acid sequence.

As used herein "an inhibitor of Band 4 activity" or "Band 5 inhibitor" is intended to include any compound, composition or environmental factor that interacts with the Band 5 protein and decreases capacitation-associated tyrosine phosphorylation of sperm proteins.

Embodiments

Proteins residing in fraction 4 of the raft plasma membrane domain of noncapacitated mouse sperm were isolated and submitted for mass spec peptide analysis. Five peptide sequences were identified by mass spec analysis of an approximately 60 kDa protein recovered from raft fraction number 4 and had the following sequence:

25- 34 CDQFVTDALK
192- 200 GLTDYSFYR
221-232 SMVGPEDAGNYRC
233- 247 CVLDTINQGHATVIR
351- 364 NASDEVKPTASGSK

An EST/cDNA seach of the existing databases revealed only one protein that contained all five fragments (See Fig. 3) and the numbers to the left of the peptide fragements indicate the location of the peptide in the identified mouse protein. Further protein and nucleic acid blast analysis identified only one hypothetical human

ortholog that matched with a significant E value to the mouse band 5 protein. A summary of the bioinformatics information generated for both the human and mouse Band 5 protein is provided in Figs. 4 & 5. An alignment of the mouse and human Band 5 sequences reveals the two proteins share a high degree of sequence similarity (See Fig. 6).

One embodiment of the present invention is directed to the mouse and human Band 5 proteins that are testis abundant and expressed predominantly if not exclusively in the male germ cells of humans and mice. More particularly the present invention is directed to mouse and human Band 5 and the use of that protein to prepare and isolate compounds that can be used as diagnostic and contraceptive agents.

The association of this unique protein with the raft membrane domains has led applicants to believe that this protein is relevant to capacitation and/or sperm/egg binding, and thus the Band 5 gene and protein product represent potential targets for of contraceptive agents. Accordingly, one aspect of the present invention is directed to the isolation of human Band 5 and its use in isolating agents that inhibit capacitation-associated tyrosine phosphorylation. Such inhibitors can then be used as contraceptive agents to inhibit fertilization. In accordance with one embodiment, the Band 5 proteins will be used to screen for specific inhibitors of Band 5 activity and these inhibitors will be used either alone or in conjunction with other contraceptive agents to prevent unintended pregnancies.

In accordance with one embodiment of the present invention a purified polypeptide is provided comprising the amino acid sequence of mouse or humand Band 5 (as shown if Fig. 6), or an amino acid sequence that differs from those sequences by one or more conservative amino acid substitutions. In another embodiment the purified polypeptide comprises an amino acid sequence that differs from those of Fig. 6 by less than 5 conservative amino acid substitutions, and in a further embodiment, by 2 or less conservative amino acid substitutions. In one embodiment the purified polypeptide comprises the amino acid sequence of Fig. 6.

The polypeptides of the present invention may include additional amino acid sequences to assist in the stabilization and/or purification of recombinantly produced polypeptides. These additional sequences may include intra- or inter-cellular targeting peptides or various peptide tags known to those skilled in the art. In one embodiment, the purified polypeptide comprises an amino acid sequence selected from Fig. 6 and a peptide tag. Suitable expression vectors for

expressing such fusion proteins and suitable peptide tags are known to those skilled in the art and commercially available. In one embodiment the tag comprises a His tag (see Example 4). In another embodiment, the present invention is directed to a purified bioactive polypeptide that comprises a portion of a polypeptide of Fig. 6.

The present invention also encompasses nucleic acid sequences that encode the polypeptides of Fig. 6. Primers were designed to an internal 500bp segment of the mouse testic cDNA for Band 5. A gene specific product was obtained by PCR. Using the gene specific primers and RACE extension of marathon ready mouse testis cDNA (Clonetech) the complete 1.5 kb insert was cloned and sequenced. The present invention is also directed to recombinant human Band 5 gene constructs. In one embodiment, the recombinant gene construct comprises a non-native promoter operably linked to a nucleic acid sequence encoding the polypeptide of Fig. 6. The non-native promoter is preferably a strong constitutive promoter that allows for expression in a predetermined host cell. These recombinant gene constructs can be introduced into host cells to produce transgenic cell lines that synthesize the Band 5 protein. Host cells can be selected from a wide variety of eukaryotic and prokaryotic organisms, and two preferred host cells are *E. coli* and yeast cells.

In one embodiment the introduced nucleic acid is sufficiently stable in the transgenic cell (i.e. incorporated into the cell's genome, or present in a high copy plasmid) to be passed on to progeny cells. The cells can be propagated *in vitro* using standard cell culture procedure, or in an alternative embodiment, the host cells are eukaryotic cells and are propagated as part of an animal, including for example, a transgenic animal. In one embodiment the transgenic cell is a human cell and comprises a nucleic acid sequence encoding the human Band 5 protein.

The present invention also encompasses a method for producing human or mouse Band 5. The method comprises the steps of introducing a nucleic acid sequence comprising a sequence that encodes humand or mouse Band 5 into a host cell, and culturing the host cell under conditions that allow for expression of the introduced human Band 5 gene. In one embodiment the promoter is a conditional or inducible promoter, alternatively the promoter may be a tissue specific or temporal restricted promoter (i.e. operably linked genes are only expressed in a specific tissue or at a specific time). The synthesizedBand 5 proteins can be purified using standard techniques and used in high throughput sceeens to identify compounds that bind to Band 5 under physiological relevant conditions and/or that inhibit capacitation associated phosphorylation of tyrosine residues of sperm proteins.

Alternatively, in one embodiment the recombinantly produced Band 5 polypeptides, or fragments thereof are used to generate antibodies against the Band 5 polypeptides. The recombinanatly produced Band 5 proteins can also be used to obtain crystal structures. Such structures would allow for crystallography analysis that would lead to the design of specific drugs to inhibit Band 5 function.

In accordance with one embodiment a composition is provided comprising a purified peptide of Fig. 6, or an antigenic fragment thereof. In one embodiment the peptide consists of the sequence of Fig. 6. The compositions can be combined with a pharmaceutically acceptable carrier or adjuvants and administered to a mammalian species to induce an immune response.

Another embodiment of the present invention is directed to antibodies specific for human or mouse Band 5. In one embodiment the antibody is a monoclonal antibody. The antibodies or antibody fragments of the present invention can be combined with a carrier or diluent to form a composition. In one embodiment, the carrier is a pharmaceutically acceptable carrier. Such carriers and diluents include sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable carrier, including adjuvants, excipients or stabilizers. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose, and related sugar solution, and glycols such as, propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

Antibodies to Band 5 polypeptides may be generated using methods that are well known in the art. In accordance with one embodiment an antibody is provided that specifically binds to a polypeptide selected from Fig. 6 or an antigenic fragment thereof. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. In addition, the antibodies can be formulated with standard carriers and optionally labeled to prepare therapeutic or diagnostic compositions.

In accordance with on embodiment of the inventon and antibody is provided that specifically binds to the peptide sequence SMVGPEDAGNYRC. This peptide is unique in the database for mouse Band 5. No other proteins match exactly to this sequence and the sequence is highly conserved between the mouse and human Band 5 sequences. This unique 13 aa sequence in the nonordinary secondary structure domain in Band 5. Results of a preliminary antibody screen are shown in

Fig. 9. One specific band is detected at about 100 kDa whereas the expected MW is 45 kDa. The preimmune and secondary antibody controls did not produce any significant signal.

To determine if the Band 5 protein is involved in sperm capacitation an experiment was conducted using Western blot analysis of sperm proteins to detect capacitation-associated tyrosine phosphorylation of sperm proteins under varying conditions. In particular proteins were isolated from sperm cells under four separate conditions:

1. Noncapacitated Sperm
2. Capacitated Sperm-1 Hour @ 37°
3. Capacitated Sperm in the Presence of a 1:50 Dilution Of Immune Sera
4. Capacitated Sperm in the Presence of a 1:50 Dilution Of Pre-Immune Sera

The results are shown in Fig. 10. Two sets of mouse sperm proteins were used for this experiment. Both sets indicate that the antibody generated against the peptide sequence SMVGPEDAGNYRC reduce the amount of tyrosine phosphorylation of sperm proteins.

Real time PCR was conducted using primers that were designed to produce a product less than 200 bp in length. An initial run was done on mouse testis quick-clone cDNA (Clonetech) using G3PHD primers as a positive control and water as a negative control. As shown in Fig. 7 a single product was produced as indicated by the melt curbve and by agarose gel analysis. Real time PCR was then conducted using a mouse multiple tissue cDNA panel screen. The results are shown in Fig. 8 and indicate that the Band 5 transcript is highly expressed in testis.

Since Band 5 is demonstrated herein to be highly testis abundant (See Figs 5-7), this makes Band 5 an optimal target for the development of drugs that modulate its activity to study Band 5's role in spermiogenesis. Furthermore, inhibitors of Band 5 activity are anticipated to have utility as contraceptive agents. In accordance with one aspect of the present invention the Band 5 protein is used as a target for the development of novel drugs. Progress in the field of small molecule library generation, using combinatorial chemistry methods coupled to high-throughput screening, has accelerated the search for ideal cell-permeable inhibitors. In addition, structural-based design using crystallographic methods has improved the ability to characterize in detail ligand-protein interaction sites that can be exploited for ligand design.

In one embodiment, the present invention provides methods of screening for agents, small molecules, or proteins that interact with polypeptides comprising the sequence of Fig. 6 or bioactive fragments thereof. The invention encompasses both *in vivo* and *in vitro* assays to screen small molecules, compounds, recombinant proteins, peptides, nucleic acids, antibodies *etc.* which bind to or modulate the activity of Band 5 and are thus useful as therapeutic or diagnostic markers for fertility.

In one embodiment of the present invention Band 5 polypeptides for Fig. 6 are used to isolate ligands that bind to Band 5 under physiological conditions. The screening method comprises the steps of contacting a Band 5 polypeptide with a mixture of compounds under physiological conditions, removing unbound and non-specifically bound material, and isolating the compounds that remain bound to the tssk polypeptide. Typically, the Band 5 polypeptide will be bound to a solid support, using standard techniques, to allow for rapid screening of compounds. The solid support can be selected from any surface that has been used to immobilize biological compounds and includes but is not limited to polystyrene, agarose, silica or nitrocellulose. In one embodiment the solid surface comprises functionalized silica or agarose beads. Screening for such compounds can be accomplished using libraries of pharmaceutical agents and standard techniques known to the skilled practitioner.

Ligands that bind to the Band 5 polypeptides can then be further analyzed for agonists and antagonists activity through the use of an *in vitro* kinase assay. Inhibitors of Band 5 associated kinase activity have potential use as agents that prevent maturation/capacitation of sperm. In accordance with one embodiment, inhibitors of Band 5 are isolated as potential contraceptive agents. Such inhibitors can be formulated as pharmaceutical compositions and administered to a subject to block spermatogenesis and provide a means for contraception.

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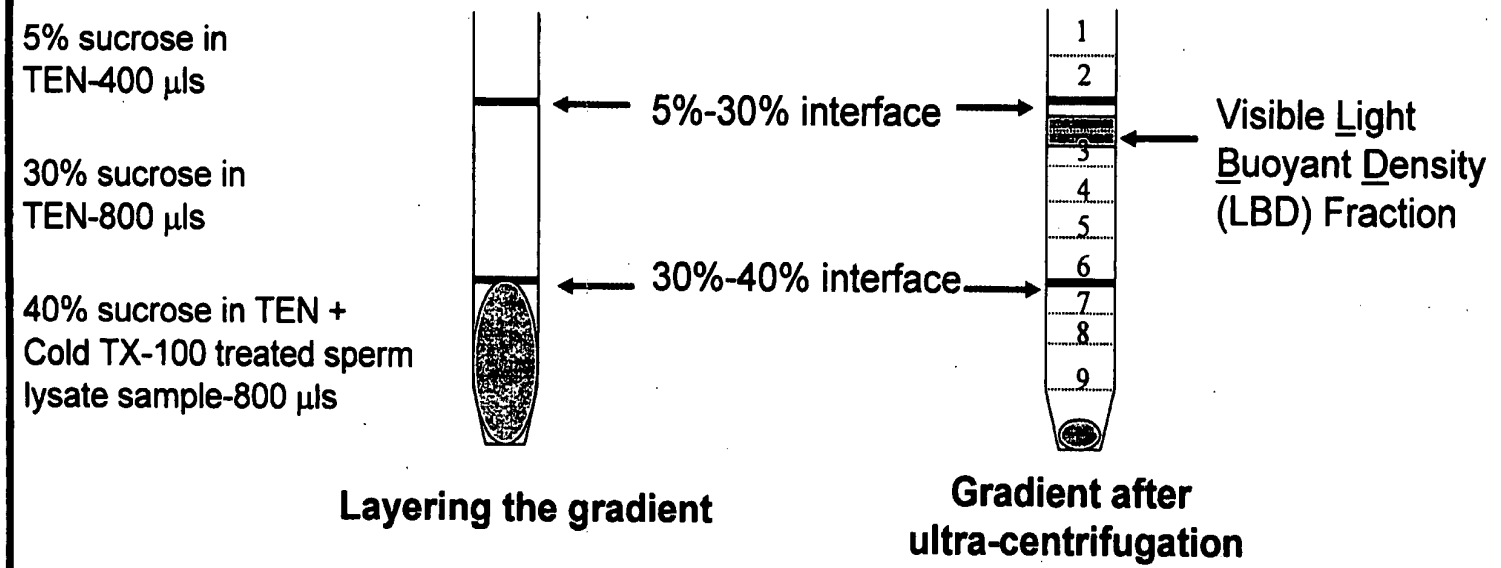
Fig 1

PREPARATION, ULTACENTRIFUGATION AND COLLECTION OF THE SUCROSE GRADIENT : VISUAL IDENTIFICATION OF LIGHT BUOYANT DENSITY (LBD) FRACTIONS

2 ml Sucrose Gradient



After centrifugation at 200,000 g for 18 hours, nine 200 µl fractions were carefully collected and prepared for 4-16% linear gradient SDS-PAGE electrophoresis. All fractions were Silver Stained for total protein detection. Also, Westerns blots were preformed to detect the Cav1α isoforms and their distribution throughout the gradient.

Fig 2

RAFT ISOLATION IN NON AND CAP MOUSE SPERM

SILVER STAIN ANALYSIS

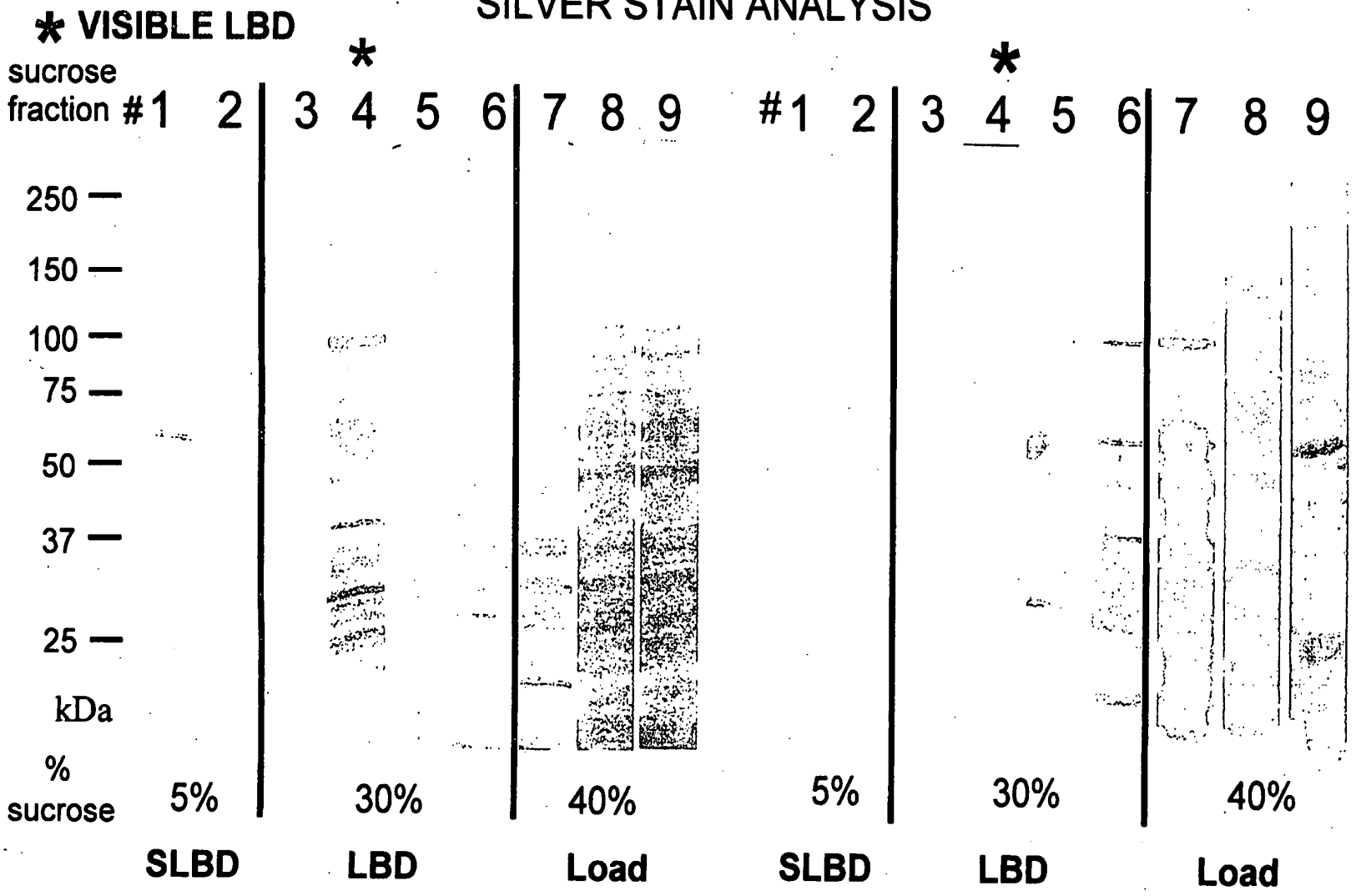


Fig 3

INITIAL MASS SPEC IDENTIFICATION OF HYPOTHETICAL PROTEIN "BAND 5"

Band 5 (KD1-39-3)
>gi|12840105|dbj|BAB24761.1| (AK006830) evidence:NAS~hypothetical protein~putative [Mus musculus] [MASS=44885]

MGPHFTLLLA ALANCLCPGR PCIKCDQFVT DALKTFENTY LNDHLPHDIH KNVMRMVNHE
VSSFGVVTSA EDSYLGAVDE NTLEQATWSF LKDLKRITDS DLKGELFIKE LLWMLRHQKD
IFNNLARQFQ KEVLCPNKCG VMSQTLIWCL KCEKQLHICR KSLDCGERHI EVHRSEDLVL
DCLLSWHRAS KGLTDYSFYR VWENSSETLI AKGKEPYLTK SMVGPEDAGN YRCVLDTINQ
GHATVIRYDV TVLPPKHSEE NQPPNIITQE EHETPVHVTP QTPPGQEPES ELYPELHPEL
YPELIPTVAQ NPEKKMKTRL LILLTLGFVV LVASIIISVL HFRKVSAKLK NASDEVKPTA
SGSKSDQSLS QQMGLKKASQ ADFNSDYSGD KSEATEN

>monoisotopic mass = 44838

BASED UPON EST/cDNA In the DATABASE

position sequence (NCBI BLAST link)

25- 34	CDQFVTDALK
192- 200	GLTDYSFYR
221-232	SMVGPEDAGNYRC
233- 247	CVLDTINQGHATVIR
351- 364	NASDEVKPTASGSK

} ACTUAL PEPTIDES ID'ED BY MASS SPEC

SMVGPEDAGNYRC BLASTP SUGGESTS PEPTIDE IS UNIQUE TO BAND 5

Fig 4

BIOINFORMATICS SUMMARY

PROTEIN and NUCLEOTIDE BLASTS

The only protein that matches with a significant E value to the mouse band 5 is a hypothetical human ortholog. Also true at the EST/cDNA level as demonstrated by the nucleotide BLASTN.

PROTEIN	aa	pMW	pI	TM	S-S	Domains	ID#
Mouse	397	44885	5.9	2	3	Ig-like	BAB24761.1
Human	350	38958	6.0	2	3	Ig-like	NP_872381

GENE	Chromosome	Exons	EST Source	Accession#
Mouse	7 B2	10	Testis cDNA, full-length insert RIKEN; 1479 bp	AK006830
Human	19q13.33	8	Adult brain medulla mRNA; 1695 bp	NM_182575

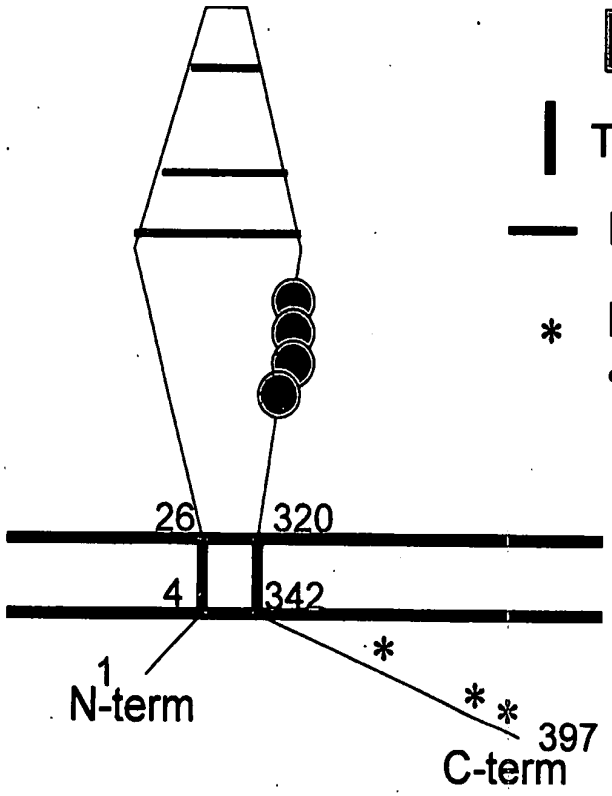
Fig 5

PROTEIN MODEL BASED ON BIOINFORMATICS

PROTEIN LEVEL

1° •397 aa-mouse from Riken testis EST evidence
•350 aa-human "hypothetical" with 65% identity to mouse
•no other significant BLASTP hits to any known proteins

2° •both mouse and human predicted to be membrane proteins with 2 trans membrane domains



● NORSS-PEPTIDE ANTIBODY-PEPTIDE IDED
SMVGPEDAGNYRC

| TRANSMEMBRANE DOMAIN~22 aa

— PREDICTED DISULFIDE BRIDGES

* POTENTIAL PHOSPHORYLATION SITES
• PKA, PKC, CKII

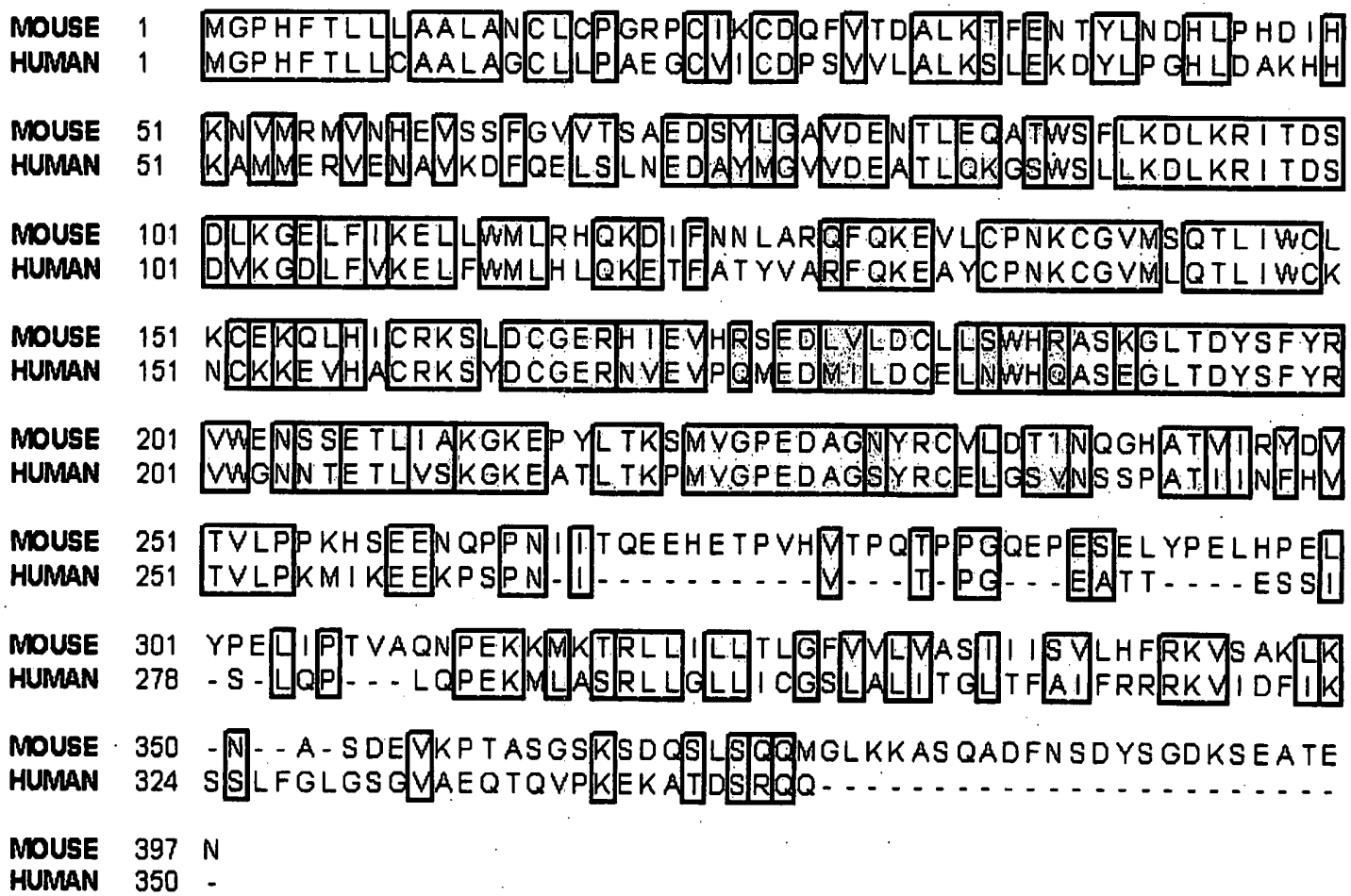
MULTIPLE POST-TRANSLATIONAL MODS PREDICTED

MOUSE SPERM PLASMA MEMBRANE

Fig 6

PAIRWISE ALIGNMENT OF MOUSE AND HUMAN BAND 5 PROTEINS

46% IDENTICAL
65% SIMILAR



AT THE cDNA LEVEL THE TWO ARE 75% IDENTICAL BY BLAST ALIGNMENT

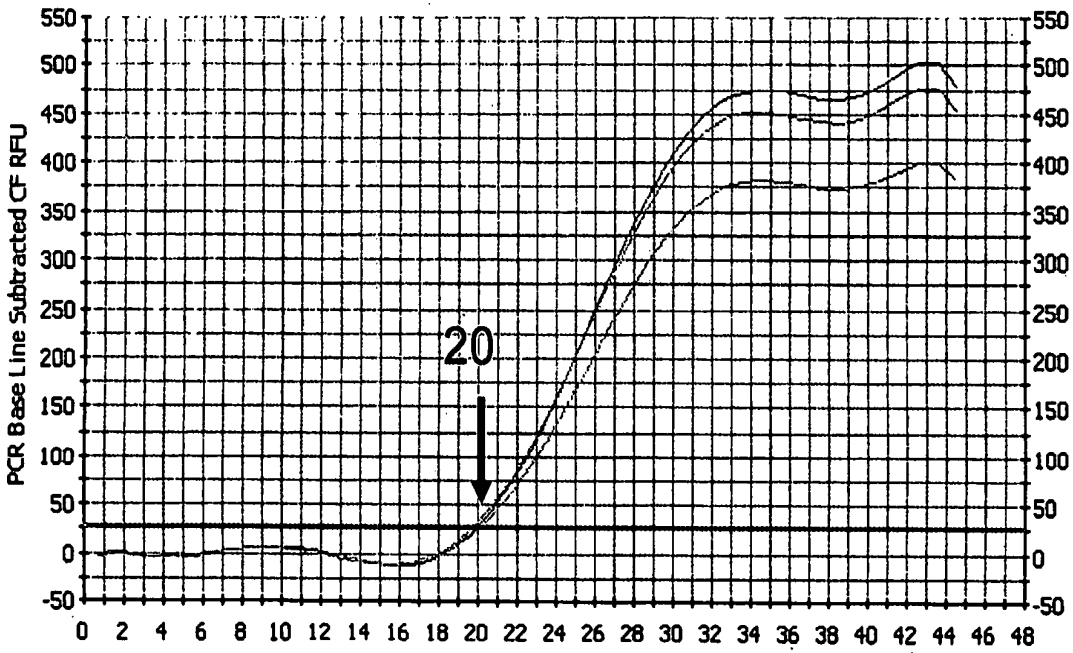
Fig 7

REAL-TIME PCR OF MOUSE TESTIS cDNA

A.

Threshold Cycle

The lower this cycle the greater The initial amount of cDNA

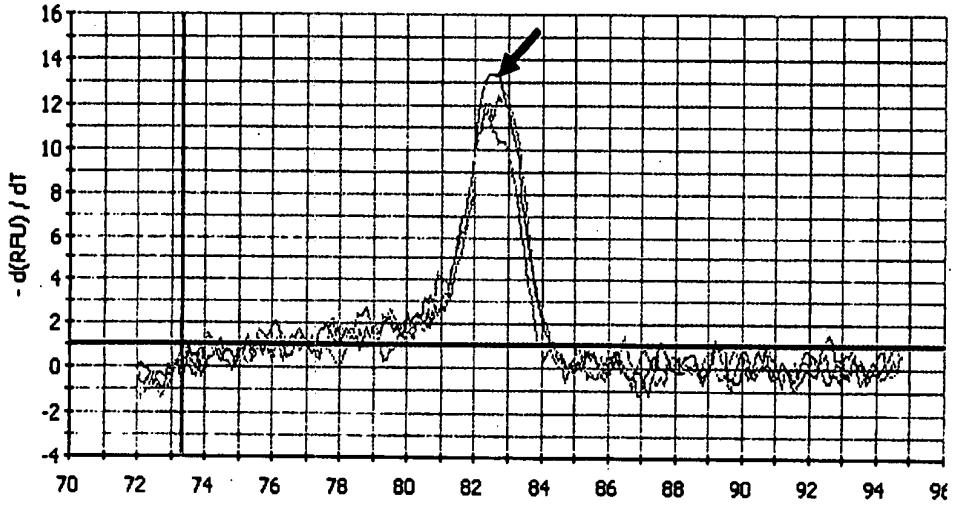


PCR cycle where product fluorescence crosses threshold

B.

Melt Curve

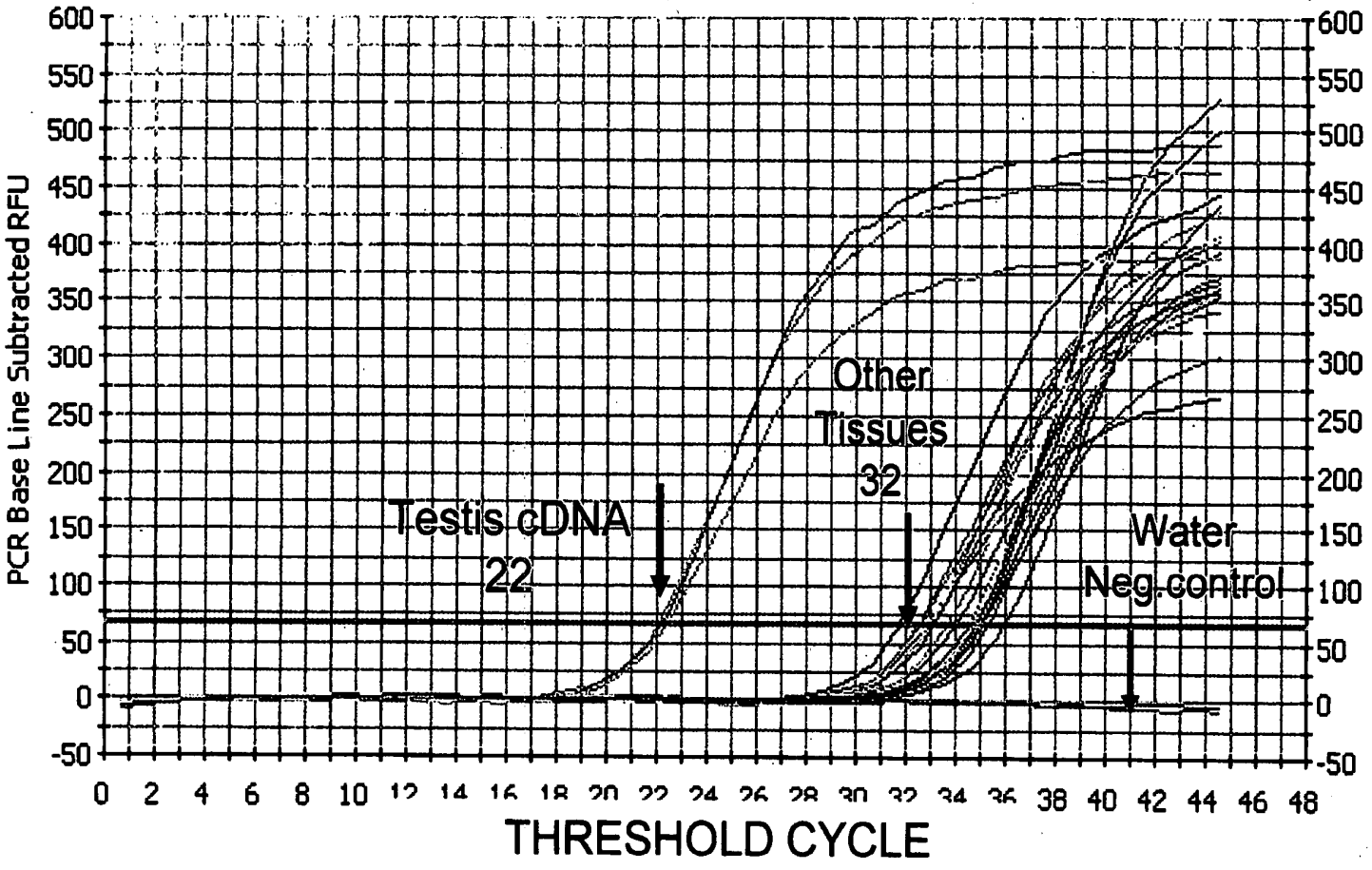
Single peak Implies single product



Temperature where product melts

Fig 8

REAL-TIME PCR MOUSE MULTIPLE TISSUE cDNA PANEL SCREEN



Significance: The Band 5 transcript is highly expressed in testis

In Other Tissues: Heart, Brain, Liver, Lung, Kidney, Pancreas, Skeletal Muscle
The threshold cycle is 10 to 14 cycles later suggesting a much lower level of transcript expression. Is this physiologically significant???

Fig 9

PRELIMINARY RESULTS WITH THE ANTI-PEPTIDE ANTIBODY

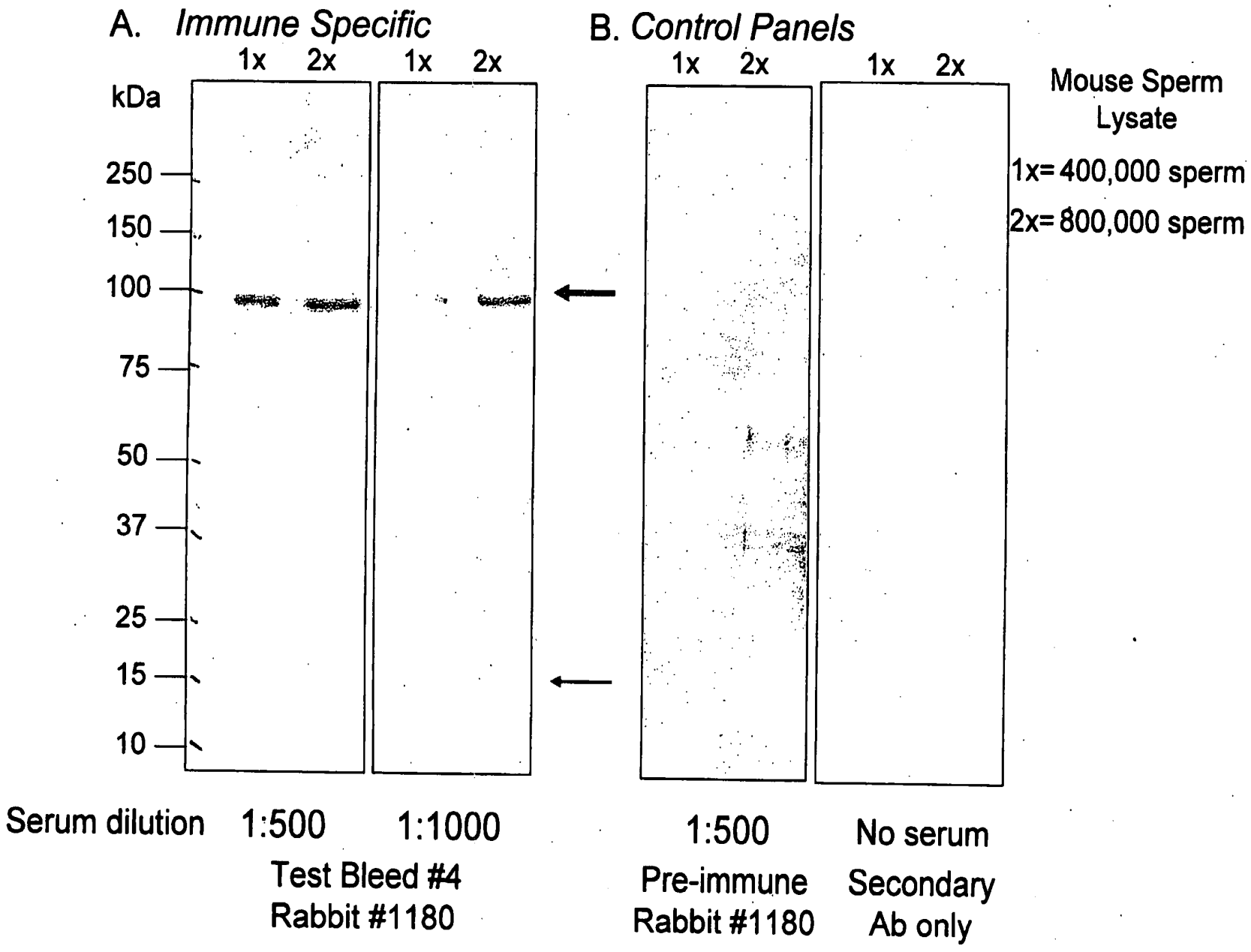


Fig 10

TYROSINE PHOSPHORYLATION OF SPERM PROTEINS, A MARKER FOR CAPACITATION:

Immune sera reduces capacitation-associated pTyr